REPRODUCTIVE BIOLOGY AND EVOLUTION IN THE PTERIDOPHYTA

ROBERT M. LLOYD[1]

In flowering plants many of the diverse characteristics utilized in systematics to determine phylogenetic relationships represent direct adaptations to specific methods of pollination or reproduction. In turn, each of these specific methods can determine to a large extent the total amount of inbreeding and outbreeding occurring and thus control population variability, evolutionary potential, and geographical distribution of the species. These phenomena and influences are well known and have been discussed extensively in the literature.

In ferns many of the diverse characteristics utilized in systematics to determine phylogenetic relationships relate to the production of spores and include the size, shape, position and development of the sorus, the sporangia, and the spores contained within. The reproductive mechanisms, however, which determine genetic diversity and evolutionary potential, are held in a free-living independent gametophyte generation, a generation which, with the exception of its morphology, has been largely ignored.

The initial modern studies on reproductive biology in ferns were done by Edward J. Klekowski, Jr., in the middle 1960's (Klekowski & Baker, 1966; Klekowski & Lloyd, 1968). Since that time numerous studies have appeared on a variety of species and phenomena by a limited number of workers (Cousens & Horner, 1970; Duckett, 1970, 1972; Ganders, 1972; Holbrook-Walker & Lloyd, 1973; Klekowski, 1969*a*, 1969*b*, 1970*a*, 1970*b*, 1970*c*, 1971*a*, 1971*b*, 1972*a*, 1972*b*, 1973*a*, 1973*b*, 1973*c*, 1973*d*; Lloyd, 1973*a*, 1973*b*, 1974; Lloyd & Klekowski, 1970; Saus, 1973; Schedlbauer & Klekowski, 1972).

Homosporous ferns for the most part produce hermaphroditic haploid gametophytes, arising from a single haploid spore by a series of mitotic divisions. As a result, gametes from a single gametophyte will be identical genetically, barring mutation. Self-fertilization, *i. e.* fusion of sperm and egg from a single gametophyte (intragametophytic selfing) will produce a zygote which is completely homozygous. Thus, in ferns it is possible to produce completely homozygous individuals in a single generation, a condition rarely achieved in flowering plants even after many generations of inbreeding. Due to the complexities of the free-living gametophyte and the genetic system of ferns, it has been necessary to utilize specific terminology to describe their mating systems. Those terms that will be used in this paper are: (1) *intragametophytic selfing*: fusion of sperm and egg from a single gametophyte, usually resulting in complete homozygosity of the resultant zygote; (2) *intergametophytic selfing*: fusion of sperm and egg from different gametophytes but both having arisen from a single sporophyte; this is analogous to inbreeding in flowering plants and results in a zygote with less heterozygosity than the parental sporophyte; (3) *intergametophytic crossing*: fusion of sperm and egg from different gametophytes, each originating from a

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[1] Department of Botany, Ohio University, Athens, Ohio 45701.

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different sporophyte; this is analogous to outbreeding in flowering plants; (4) *intergametophytic mating*: fusion of sperm and egg from different gametophytes but origin of gametophytes not specified.

Because of the unique ability of fern gametophytes to form completely homozygous zygotes it is possible to screen sporophytes for deleterious recessive genes (genetic load). Data of this type, when used in conjunction with gametophyte and gametangia ontogeny, chromosome studies, and analysis for apogamy and vegetative reproduction, has allowed us to sample populations of plants and determine their mating systems and the degree of genetic heterozygosity for deleterious lethals. With data of this type it is now possible to extrapolate further to predictions of population variability and evolutionary potential of taxa and to correlate in some cases specific mating systems with specific ecological requirements.

In the following discussion I wish to cover four specific areas of fern reproductive biology which are important and significant in evolution. These areas are (1) levels of genetic load which have been found in natural populations of ferns; (2) homoeologous chromosome pairing; (3) specific studies utilizing ferns from a variety of habitats in Hawaii to illustrate the correlations which can be made between mating systems, subsequent genetic heterozygosity, and habitat selection; and (4) allelopathic compounds and antheridogen.

Unfortunately, time does not allow me to cover equally important facets of fern biology such as apogamy and hybridization (see DeBenedictis, 1969, for review of literature on apogamy in ferns).

1. Genetic Load

Sampling for deleterious recessive genes in sporophytes is a relatively simple procedure involving the isolation of 30 or more prothalli from a single sporophyte before the attainment of sexual maturity, growing these prothalli to maturity, analysing them for the hermaphroditic condition, and watering to insure an aqueous medium for the transference of sperm to egg. More specific genetic tests have been devised (pairs/isolates test) to provide additional support for the genetic basis of the results (Klekowski, 1971*a*). Interpretation of the data from the isolate cultures is somewhat more difficult. In general, lack of sporophyte production on any hermaphroditic gametophyte, with supporting evidence from the pairs/isolates test, or visible signs of sporophytic abortions or malformations, has been used as indicative of homozygous deleterious recessive gene combinations. From data of this type it is possible to determine in individual sporophytes heterozygosity for recessive lethals and the frequency of such sporophytes in given populations. Utilizing other morphological criteria, such as gametangia sequence of development and placement, presence or absence of polyembryony and antheridogen, and length of generation time, it is then possible to extrapolate with a fair degree of accuracy to the primary mode of mating system utilized in nature by the species and the expected resulting degrees of heterozygosity.

Genetic load studies which have been published on ferns from natural populations indicate that about 16 species of the more or less 10,000 species of

TABLE 1. Genetic load studies in ferns from natural populations. Given as percent of gametophytes bearing recessive sporophytic lethal genes, per sporophyte sampled.

Species	Genetic load (mean)	Source
Ceratopteris thalictroides	0.0–6.0%	Klekowski, 1970*a*
Cibotium chamissoi	26.6–90.0 (59.0)%	Lloyd, 1974
Dicranopteris linearis	20.0–65.0 (36.6)%	Lloyd, 1974
Microsorium scolopendria	0.0–10.0 (7.2)%	Lloyd, 1974
Nephrolepis exaltata	0.0%	Lloyd, 1974
Onoclea sensibilis	6.0–41.0 (26.0)%	Ganders, 1973
O. sensibilis	0.0–45.0 (13.7)%	Saus, 1973
Osmunda regalis	5.0–100.0 (59.5)%	Klekowski, 1970*b*
Pteridium aquilinum subsp. *aquilinum*		
var. *decompositum*	6.0–72.0 (31.0)%	Klekowski, 1972*a*
var. *latiusculum*	14.6–28.0 (21.3)%	Klekowski, 1972*a*
var. *pubescens*	47.1–100.0 (64.2)%	Klekowski, 1972*a*
P. aquilinum subsp. *caudatum*		
var. *arachnoideum*		
Galapagos	0.0–19.0 (9.0)%	Klekowski, 1972*a*
Peru	67.5%	Klekowski, 1972*a*
Costa Rica/Honduras	23.6–40.0 (30.1)%	Klekowski, 1972*a*
var. *esculentum*	82.0–87.8 (84.9)%	Klekowski, 1972*a*
Sadleria cyatheoides	44.0%	Holbrook-Walker & Lloyd, 1973
S. souleyetiana	44.0%	Holbrook-Walker & Lloyd, 1973
Thelypteris palustris	30.0–92.4 (40.0)%	Ganders, 1973
Woodwardia fimbriata	0.0%	Klekowski, 1969*b*

Pteridophyta have been investigated (Table 1). However, population studies sampling large numbers of sporophytes have been done only on *Ceratopteris thalictroides* from Hawaii, *Cibotium glaucum* from Hawaii, *Onoclea sensibilis* from Ohio, and *Osmunda regalis* from Massachusetts. Mean levels of genetic load which have been found in sporophytes in nature vary from 0.0% in *C. thalictroides* and *Nephrolepis exaltata* from populations in Hawaii to over 59.0% in *Cibotium chamissoi* and *Osmunda regalis*. The possible significance of these levels will be discussed below. The mean genetic load for all species studied to date, if such a figure has any significance, is about 29.4%.

Frequency of genetic load in sporophytes of ferns is dependent upon a large number of factors including polyploidy, mutation rate, mating system, population size, habitat diversity, and natural selection. Mating system has a large influence on levels of genetic load. Populations with high frequencies of intergametophytic mating can be shown to consistently express genetic load, whereas populations with primarily intragametophytic mating express little or none. Lloyd (1974) has shown in *Nephrolepis exaltata* that 100% of the sporophytes tested from recent lava flows on Hawaii exhibited no genetic load. Characteristics of gametangia ontogeny, with large numbers of antheridia being produced before archegonia and continuously throughout archegonial production, indicates that the mating system for this species is probably one of intragametophytic selfing.

Klekowski (1970*a*) has shown that presence of antheridia in the interior

TABLE 2. Genetic load in *Cibotium chamissoi* relative to gametophyte gametangia ontogeny.

Ontogeny	Per cent of sporophytes exhibiting ontogeny	Genetic load (mean)
Male to hermaphroditic	30.0%	26.6–30.0 (27.8)%
Male and Female to hermaphroditic	20.0%	83.7–90.0 (86.9)%
Female to hermaphroditic	50.0%	40.0–83.7 (65.1)%

surface position prior to and during initiation of archegonia in *Ceratopteris thalictroides* can be correlated with the lack of genetic load in one Hawaiian population of this species. In species with gametangia sequences from female to hermaphroditic most genetic studies have indicated the presence of genetic load: in *Onoclea sensibilis* the mean genetic load is 13.7% (Saus, 1973); in *Sadleria cyatheoides*, 44.0% (Holbrook-Walker & Lloyd, 1973); and in *Dicranopteris linearis*, 36.6% (Lloyd, 1974). The most striking evidence for the significance of mating system and gametangia ontogeny in the level of genetic load comes from recent work on *Cibotium glaucum* (Lloyd, 1974). This is the dominant understory species in the mature rainforest on the slopes of Mauna Loa in Hawaii. Investigations of the reproductive biology of this species indicate that different sporophytes from the same population will exhibit different levels of genetic load and that these levels are correlated with the sequence of gametangia ontogeny expressed by their gametophytes (Table 2). For example, sporophytes which produce gametophytes which undergo a male to hermaphroditic ontogeny exhibit a 26.6–30.0 (mean = 27.8) % genetic load; whereas sporophytes which produce gametophytes with a female to hermaphroditic ontogeny exhibit a 40.0–83.7 (mean = 65.1) % genetic load. Sporophytes which produce gametophytes which are initially unisexual and male and female have higher levels varying from 83.7–90.0 (mean = 86.9) %. Thus, these data indicate that fewer recessive deleterious genes are found in those sporophytes which produce gametophytes which have higher probabilities of intragametophytic selfing due to their gametangia sequence; conversely, gametangia sequences favoring intergametophytic mating are produced by gametophytes from sporophytes with higher frequencies of recessive deleterious genes.

However, the correlations of mating system, gametangia ontogeny, and genetic load as presented above are too simplistic. For example, *Onoclea sensibilis* exhibits a gametangia sequence which heavily favors intergametophytic mating, with the archegoniate stage lasting from 40 to about 85 days before hermaphroditism is attained. In addition, Näf *et al.* (1969) have documented the possible production of an antheridogen by gametophytes of this species. In dense cultures younger prothalli from later germinating spores will precociously produce antheridia. Some of these gametophytes remain male throughout their life span (Lloyd, 1971; Saus, 1973). Although the species appears to have high probabilities of intergametophytic mating, the genetic load found in sporophytes is relatively low (mean = 13.7%). In *Microsorium scolopendria* a similar situation is encountered. This species produces gametophytes with a gametangia sequence from female to hermaphroditic with the archegoniate stage lasting from 14 to 59 days. However, genetic load is low (0–10%, mean = 7.2%). In this latter species one must consider population size as well as gametangia ontogeny. Five plants forming the entire population were sampled from a lava flow dated from 1750 on the island of Hawaii. It is not unlikely that initial establishment of the species on the flow was by spores blown in by the wind, germinating to produce gametophytes which were most likely sufficiently isolated from one another to prevent intergametophytic mating. Thus, sporophytes resulting from these gametophytes would be the result of intragametophytic selfing and therefore homozygous and without deleterious recessive genes. Other sporophytes found here could be the product of the initial or subsequent introductions or more than likely of spores produced by the original pioneer. If the latter is true, lethals present in these plants would most likely be the result of accumulated mutations. In *Ceratopteris thalictroides*, the original population tested by Klekowski (1970*a*), in which he found no genetic load, was relatively small, consisting of plants found in only a few taro patches on the island of Oahu. Lloyd (1973*a*) has found that sexual reproduction in this population ceases between ten and 13 months after establishment and that a significant amount of reproduction takes place by vegetative budding. In a larger population of the same species from Kauai (probably introduced there from Oahu) Klekowski has found recessive deleterious genes in 55.5% of the sporophytes tested which varied from 0.0–7.5 (mean = 3.0) % (unpublished personal communication). In *Osmunda regalis*, Klekowski (1970*b*) found that the largest population tested had the highest genetic load. In addition, this species generally exhibits gametangia ontogeny from male to hermaphroditic yet exhibits high levels of genetic load. He has concluded that *O. regalis* has a gametophyte morphology which promotes intragametophytic selfing when gametophytes are sparse and intergametophytic mating when they are abundant.

A further influence on genetic load in natural populations is the availability of habitats for gametophyte establishment, prolonged growth, and survival. In general, longer lived gametophytes tend to favor intergametophytic mating whereas shorter generations favor intragametophytic selfing (Klekowski, 1969*a*). Klekowski (1972*a*) has shown in *Pteridium aquilinum* that plants from the Galapagos Islands exhibit a mean genetic load of 9.0%, whereas plants of the same species from Hawaii exhibit a mean genetic load of 31.0%. He interpretes this to be perhaps a response of the latter populations having greater genetic diversity brought about by more favorable, diverse mesic habitats with subsequent establishment of intergametophytic mating and interlocus heterozygosity. The Galapagos Islands, on the other hand, are more xeric and less diverse, a situation which would favor more shortened gametophyte generations, less genetic diversity, and apogamy. The populations of *P. aquilinum* occurring there probably arrived by spores from South America and probably initially produced sporophytes by intragametophytic selfing. Thus, they are more depauperate genetically, with a greater probability of an intragametophytic mating system and the resulting lowered genetic load.

2. Homoeologous Chromosome Pairing

One of the significant new hypotheses to come out of the study of fern biology during this century has been the recent documentation of homoeologous chromosome pairing in *Ceratopteris* by Edward Klekowski, Jr., of the University of Massachusetts, and its possible relationship to the high levels of polyploidy found within the Pteridophyta (Klekowski & Baker, 1966; Klekowski, 1972 *b*, 1973*c*, 1973*d*). He has related the evolution of polyploidy in homosporous ferns to suspected high frequencies of intragametophytic selfing due to the homothallic hermaphroditic nature of most gametophytes as a means of storing genetic diversity by duplicated alleles regardless of the subsequent homozygosity of sporophytes produced by the mating system. His data clearly indicate that ferns with an obligate intergametophytic mating system (*e. g.* heterosporous ferns) have chromosome numbers (mean $n = 13$) more nearly approaching those of flowering plants, whereas homosporous ferns have numbers which average considerably higher (mean $n = 54$). In addition, 40% of homosporous ferns show additional polyploidy over base numbers which themselves may be of polyploid origin. For example, in *Osmunda*, which has a haploid number of 22, Tatuno and Yoshida (1966, 1967) have shown that this probably represents an ancient tetraploid condition based on $x = 11$. From these studies and others it can be concluded that polyploidy is a major phenomenon present in homosporous ferns and that taxa with duplicated chromosome sets have had the greatest evolutionary potential (Klekowski, 1973*c*).

Duplicated chromosome sets allow for the storage of genetic material even in homzygous ferns. Mutations can occur in one allele without affecting another or required physiological processes, thus producing interlocus heterozygosity. In ferns which normally undergo intragametophytic selfing, recombination could occur by occasional intergametophytic crossing producing heterozygous sporophytes, followed by meiosis.

If, however, homoeologous chromosome pairing (pairing between duplicated chromosomes within polyploid sets) is occurring, species which normally undergo intragametophytic selfing can produce heterozygous spores from homozygous spore mother cells (if interlocus heterozygosity is present) rather than the expected homozygous spores produced by homologous pairing. Therefore, polyploidy, followed by mutation, serves to store genetic diversity; homoeologous pairing, when present, serves to release that variability even in ferns which frequently undergo intragametophytic selfing. This theory can be extrapolated to include most apogamous ferns which produce spores by meiosis.

Klekowski's evidence for homoeologous pairing has come from his initial observations of morphological variation in homozygous families of sporophytes to marker genes and cytological manifestations of paracentric inversions and translocations in completely homozygous sporophytes. These latter aberrations would not be visible in sporophytes with homologous pairing. This phenomenon has been documented only in *Ceratopteris*, and whether it occurs in any other species remains to be seen.

3. REPRODUCTIVE BIOLOGY AND HABITAT SELECTION

Recent studies on Hawaiian ferns have brought to light several distinct correlations between reproductive biology, habitat preference, and geographical distribution in *Sadleria* spp. (Holbrook-Walker & Lloyd, 1973). In this genus it has been possible to characterize the mating system of three of the four species and to relate these mating systems in some cases to specific abilities (or lack of) of the species to colonize lava flow habitats. Of the four species (all of which are endemic to Hawaii) *S. cyatheoides* is the most widely distributed in terms of ecology and geography and is the only species found as a colonizer on new lava flows. This species is characterized by an intergametophytic mating system with gametangia ontogeny (initially bigametophytic or female to hermaphroditic) which allows for possible intragametophytic selfing (hermaphroditism and polyembryony) with time. As plants found on lava are sometimes widely distributed they probably initially result from single spore introductions and the obligate intragametophytic selfing following. The second most common species, *S. pallida*, is never found as a colonizer and usually only occurs in relatively dense populations in stable mature forest vegetation complexes. Mating system studies in this species indicate that it may have a nearly obligate intergametophytic mating system due to the non-synchronous production of archegonia and antheridia. In our studies only 4% of the isolate culture and 25% of the composite culture gametophytes produced sporophytes, indicating that their was at least minimal overlap of the two gametangia in some gametophytes. Morphological studies indicated that archegonia production ceases with the initial production of antheridia or shortly thereafter. This hiatus in gametangia production appears to be significant as one possible cause for restricting the species to areas with high gametophyte densities, in other words, to habitats with a significant number of other mature sporophytes present.

A third species, *Sadleria squarrosa*, is restricted to extremely wet, deeply shaded steep banks and is rarely found scattered throughout the rain forest. Gametophytes of this species were found to be intolerant of light intensities used in our culture conditions (ca. 250 ft.c.). Lowering of the light intensity to less than 100 ft.c., however, allowed gametophytes to more normally complete their ontogeny. Similar phenomena were observed in gametophytes of *Onocleopsis hintonii*, a species found in only a few dark, wet canyons in southern Mexico and Guatemala (Lloyd, 1971). Conversely, gametophytes of the taro-patch-inhabiting *Ceratopteris thalictroides*, a habitat with high temperatures and light intensities, grow best and produce sporophytes at a more rapid rate at 500 ft.c. or greater and at temperatures above 33° C. At temperatures of 18° C. spore germination and gametophyte growth is greatly retarded (Lloyd, unpublished).

From these studies it is apparent that the gametophyte generation has as specific habitat requirements and responses as the sporophytes which produced them.

Further studies on Hawaiian ferns have elucidated trends in mating system and subsequent heterozygosity for genetic load between species found as dominants on lava flows and those found as dominants in mature rain forest

TABLE 3. Genetic load studies in Hawaiian sporophytes: mean percent of gametophytes bearing recessive lethal genes per population.

Species	Number gametophytes studied	Mean percent bearing lethal genes
Nephrolepis exaltata (pioneer lava)	100	0.0%
Microsorium scolopendria	110	7.2%
Sadleria cyatheoides	75	44.0%
Dicranopteris linearis	60	36.0%
Sadleria souleyetiana	75	44.0%
S. pallida	75	96.0%[a]
Cibotium chamissoi	270	59.0%

[a] Lack of sporophyte production probably due to non-synchronous gametangia production.

(Lloyd, 1974). Tables 3, 4, and 5 summarize these data. The dominant species found as a pioneer on lava is *Nephrolepis exaltata,* appearing as soon as 4.5 months after a flow. This habitat is almost uniformly depauperate in terms of soil, diversity, and other higher plants. Studies on *N. exaltata* indicate a mating system with high probabilities of intragametophytic selfing and no genetic load. Furthermore, this species reproduces profusely by vegetative stolons, is incapable of tolerating shade, and after establishment of tree cover will die out. Other species found in this habitat, which have been tested, also show little genetic heterozygosity for deleterious recessive genes, although their mating systems can be variously characterized as intra- or intergametophytic. On the other hand, dominants in the mature rain forest (*Cibotium chamissoi, Sadleria pallida*) can be characterized by much higher levels of genetic load and by intergametophytic mating systems.

Population size has been shown to affect heterozygosity by reducing the opportunities for intergametophytic mating. In some pioneer species on lava, such as *Microsorium scolopendria,* this is apparently the case. In others, however, such as the dominant *Nephrolepis exaltata,* the population consists of hundreds of plants. In the mature rain forest, density of *Cibotium chamissoi* is very high. As plants of this species produce large numbers of spores simultaneously when fertile it appears that gametophyte densities are probably also very high, thus increasing probabilities for intergametophytic mating which is reflected in the higher levels of heterozygosity for recessive deleterious genes.

Two factors may play a part in the intragametophytic mating system found

TABLE 4. Genetic load of dominant species relative to pioneer and non-pioneer habitats in Hawaiian ferns.

Habitat	Genetic load (mean)
Pioneer lava flows	0.0–44 (17.0)%
Intermediate forest (young)	10–59 (37.2)%
Mature rain forest	44–59 (51.5)%[a]

[a] Data for *Sadleria pallida* are not included.

TABLE 5. Age of appearance of species, vegetation type, and probable mating system of Hawaiian ferns.

Species	Pioneer habitats 1971–1950–1850–1750	Intermediate forest	Mature forest	Mating system
Nephrolepis exaltata	———————			Intragametophytic
Microsorium scolopendria	—————			Intra/intergametophytic
Psilotum nudum	——	———		Probably intragametophytic
Polypodium pellucidum	———————	— — — — —	— —	Probably intragametophytic
Sadleria cyatheoides	— — — — ———	———		Intergametophytic
Dicranopteris linearis	— — — — —	———		Intergametophytic
Cibotium chamissoi	— — —	———	———	Intergametophytic
Sadleria pallida			———	Intergametophytic
S. souleyetiana			———	Intergametophytic
S. squarrosa			— — —	Probably intragametophytic

in *Nephrolepis exaltata.* The first would relate to shortening the gametophyte generation to facilitate reproduction in what appears to be a harsh xeric environment. The second factor relates to the actual genotype of the plants, which by the process of intragametophytic selfing should be relatively homozygous. In the first instance, the harsh environment of the barren lava flow is restricted to the edaphic factor as soil profiles are lacking. Nearly all sporophytes observed were growing in shallow to deep, wet, cool crevices. With the moderate rainfall in these regions it would appear that these crevices would provide gametophytes with substantial moisture for a lengthy growing season. However, if this is the case, one would expect to find more variety in the species found in this habitat. The second factor, that of genetic homozygosity, deserves more consideration. In this case, intragametophytic selfing can be seen as a mechanism whereby this species can maintain favorable gene combinations which are opportunistic for pioneering and survival on lava flows.

4. Allelopathic Compounds and Antheridogen

Recently, Davidonis and Ruddat (1973) and Petersen and Fairbrothers (1973) have discovered allelopathic compounds produced by sporophytes which detrimentally affect gametophyte growth and development. Davidonis and Ruddat observed that gametophytes of *Thelypteris normalis* would not grow under sporophytes of that species. They determined that the roots of this species were releasing two groups of allelopathic compounds, thelypterin A and B, which were biologically active. Thelypterin A was characterized as a secondary amine-containing heterocyclic molecule with an anionic function. Further experiments utilizing gametophytes of *Pteris longifolia* and *Phlebodium aureum* indicated similar responses to the compounds.

Petersen and Fairbrothers have shown that gametophytes of *Osmunda* spp. are allelopathic to gametophytes of *Dryopteris* spp. by halving the growth rate and cell size of the latter. Both of these studies have attempted to relate ecological advantages in the production of these compounds by the elimination of competitive sporophytes.

Antheridogen has been reported in several species of unrelated ferns, including *Onoclea sensibilis* (Näf *et al.*, 1969), *Ceratopteris thalictroides* (Schedlbauer & Klekowski, 1972), *Pteridium aquilinum* (Döpp, 1950), *Anemia phyllitidis* (Näf, 1959), and *Lygodium japonicum* (Näf, 1960). Antheridogen functions in precociously initiating antheridia on slower growing and later germinating spores and gametophytes of the same species. This phenomenon has been interpreted as a mechanism for increasing the probabilities of intergametophytic mating by increasing the number of intergametophytic sperm present in any spatially dense gametophyte colony. In some cases antheridogen has been shown to precociously induce antheridia formation on gametophytes of unrelated ferns. For example, antheridogen A, released by *Pteridium,* affects gametangia ontogeny in many species of Adiantaceae, Davalliaceae, Aspidiaceae, Blechnaceae, and in one species of Dennstaedtiaceae (Voeller, 1964). The biological significance of this, if any, has yet to be shown conclusively.

TABLE 6. Gametophyte characteristics and predicted mating system.

Intragametophytic	Intergametophytic
Male to hermaphroditic gametangia ontogeny	Female to hermaphroditic; unisexual; heterosporous
Archegonial necks curved toward antheridia	Archegonial necks curved away from antheridia
Simple polyembryony absent	Simple polyembryony present
Underground gametophytes	Superficial gametophytes
Antheridogen absent	Antheridogen present
Generation time short	Generation time long
Genetic load absent	Genetic load present
Apogamy present	Apogamy absent
Vegetative reproduction present	Vegetative reproduction absent
Low recombinational index	High recombinational index
Low hybridization index	High hybridizational index
Narrow ecological tolerance	Wide ecological tolerance

CONCLUSIONS

We have seen from the above discussion that there are numerous parameters affecting reproduction, diversity, and evolution in ferns. These parameters vary from polyploidy, which stores genetic variability, to the mating system, which either releases some of this variability by intergametophytic mating and recombination or perpetuates homozygosity by intragametophytic selfing. Factors directly affecting mating systems in ferns are gametangia ontogeny, production of antheridogen, and genetic load. Genetic load in itself can be used not only as an indicator of heterozygosity in natural populations but can function in supporting intergametophytic mating by eliminating products of self-fertilization. Basic to the level of heterozygosity found in spores produced by a single sporophyte, however, is the presence or absence of homoeologous chromosome pairing during meiosis.

Table 6 indicates features of gametophyte biology which favor particular mating systems. Table 7 summarizes those features which would create under theoretical conditions maximum homozygosity and maximum heterozygosity in natural populations. From these summaries it can be seen that features yielding maximum heterozygosity in sexually reproducing ferns under ideal conditions include large population size, intergametophytic crossing, polyploidy, high mutation rate (assuming non-deleterious mutations), and homoeologous chromosome pairing. Characteristics favoring homozygosity include small populations, intragametophytic selfing, low polyploidy and mutation rate, and homologous pairing. It is doubtful if either of these two theoretical conditions are achieved in nature by any sexually reproducing species. It is more likely that most ferns fall somewhere in between.

Characteristics of the mating system can also influence species patterns. For example, the most distinct species in *Sadleria* (*S. squarrosa*) probably possesses an intragametophytic mating system. Other species in this genus (which have been characterized as having intergametophytic mating systems) show partial

TABLE 7. Characteristics yielding maximum homozygosity and maximum heterozygosity in natural populations of ferns.

Homozygosity	Heterozygosity
Intragametophytic selfing mating system	Intergametophytic crossing mating system
Apogamy present	Apogamy absent
Vegetative reproduction present	Vegetative reproduction absent
Small population size	Large population size
Low polyploids	High polyploids
Homologous chromosome pairing	Homoeologous chromosome pairing
Genetic load absent	Genetic load present
Low mutation rate	High mutation rate

intergradation from one taxon to the next. These patterns may have been brought about by interspecific hybridization which is facilitated by intergametophytic mating systems. *Dryopteris* in the Appalachian Mountains has evolved a number of distinct taxa through hybridization (Wagner, 1970), and work by Cousens and Horner (1970) indicate that these species may also possess intergametophytic mating systems.

Species with intragametophytic mating systems which have maintained the possibilities for intergametophytic crossing can present diversity patterns similar in many respects to those of apomictic flowering plant species which are occasionally sexual. Intragametophytic selfing, like apomixis, is a built-in mechanism for perpetuating specific genotypes. The intrinsic properties of the intragametophytic mating system, like those of apomixes, may in fact far exceed those of intergametophytic mating.

In conclusion I wish to state that it is only through understanding the basic causal factors for genetic diversity and reproduction in ferns that we will ever understand their mode of speciation and evolution. Specifically, they are those factors which are operable from the spore mother cell of the parental sporophyte, through the gametophyte, to establishment of the young sporophytic progeny. During this portion of the life-cycle, dissemination of spores takes place, genetic diversity of progeny is determined, and hybridization and apogamy occur. We are rapidly becoming aware that gametophytes require specific culture conditions for optimum growth. There are numerous parameters of gametophyte biology which have as yet not been discovered, and it is likely that many of these will be uncovered only through the investigation of gametophyte populations in nature.

LITERATURE CITED

COUSENS, M. I. & H. T. HORNER. 1970. Gametophyte ontogeny and sex expression in *Dryopteris ludoviciana*. Amer. Fern Jour. 60: 13–27.

DAVIDONIS, G. H. & M. RUDDAT. 1973. Allelopathic compounds, Thelypterin A and B in the fern *Thelypteris normalis*. Planta 111: 23–32.

DEBENEDICTIS, V. M. 1969. Apomixis in ferns with special reference to sterile hybrids. Unpublished doctoral dissertation, Univ. of Michigan, Ann Arbor.

DÖPP, W. 1950. Eine die Antheridienbildung bei Farnen fördernde Substanz in den Prothallien von *Pteridium aquilinum* (L.) Kuhn. Ber. Deutsch. Bot. Ges. 63: 139–147.

DUCKETT, J. G. 1970. Sexual behaviour of the genus *Equisetum*, subgenus *Equisetum*. Bot. Jour. Linn. Soc. 63: 327–352.

———. 1972. Sexual behaviour of the genus *Equisetum*, subgenus *Hippochaete*. Bot. Jour. Linn. Soc. 64: 87–108.

GANDERS, F. R. 1972. Heterozygosity for recessive lethals in homosporous fern populations: *Thelypteris palustris* and *Onoclea sensibilis*. Bot. Jour. Linn. Soc. 65: 211–221.

HOLBROOK-WALKER, S. & R. M. LLOYD. 1973. Reproductive biology and gametophyte morphology of the Hawaiian fern genus *Sadleria* (Blechnaceae) relative to habitat diversity and propensity for colonization. Bot. Jour. Linn. Soc. 67: 157–174.

KLEKOWSKI, E. J., JR. 1969*a*. Reproductive biology of the Pteridophyta. II. Theoretical considerations. Bot. Jour. Linn. Soc. 62: 347–359.

———. 1969*b*. Reproductive biology of the Pteridophyta. III. A study of the Blechnaceae. Bot. Jour. Linn. Soc. 62: 361–377.

———. 1970*a*. Reproductive biology of the Pteridophyta. IV. An experimental study of mating systems in *Ceratopteris thalictroides* (L.) Brongn. Bot. Jour. Linn. Soc. 63: 153–169.

———. 1970*b*. Populational and genetic studies of a homosporous fern—*Osmunda regalis*. Amer. Jour. Bot. 57: 1122–1138.

———. 1970*c*. Evidence against self-incompatibility and for genetic lethals in the fern *Stenochlaena tenuifolia* (Desv.) Moore. Bot. Jour. Linn. Soc. 63: 171–176.

———. 1971*a*. Ferns and genetics. BioScience 21: 317–322.

———. 1971*b*. Evidence for a duplicated locus in the fern *Osmunda regalis*. Jour. Heredity 62: 367–370.

———. 1972*a*. Evidence against genetic self-incompatibility in the homosporous fern *Pteridium aquilinum*. Evolution 26: 66–73.

———. 1972*b*. Genetical features of ferns as contrasted to seed plants. Ann. Missouri Bot. Gard. 59: 138–151.

———. 1973*a*. Genetic load in *Osmunda regalis* populations. Amer. Jour. Bot. 60: 146–154.

———. 1973*b*. Genetic endemism of Galapagos *Pteridium*. Bot. Jour. Linn. Soc. 66: 181–188.

———. 1973*c*. Sexual and subsexual systems in homosporous pteridophytes: a new hypothesis. Amer. Jour. Bot. 60: In Press.

———. 1973*d*. Homoeologous chromosome pairing in *Ceratopteris*. Amer. Jour. Bot. 60 (4, suppl.): 31.

——— & H. G. BAKER. 1966. Evolutionary significance of polyploidy in the Pteridophyta. Science 153: 305–307.

——— & R. M. LLOYD. 1968. Reproductive biology of the Pteridophyta, I. General considerations and a study of *Onoclea sensibilis* L. Jour. Linn. Soc. Bot. 60: 315–324.

LLOYD, R. M. 1971. Systematics of the onocleoid ferns. Univ. California Pub. Bot. 61: 1–94.

———. 1973*a*. Systematics of the genus *Ceratopteris* (Parkeriaceae), I. Sexual and vegetative reproduction in Hawaiian *Ceratopteris thalictroides*. Amer. Fern Jour. 63: 12–18.

———. 1973*b*. Facultative apomixis and polyploidy in *Matteuccia orientalis* (Hook.) Trev. Amer. Fern Jour. 63: 43–48.

———. 1974. Mating systems and genetic load in pioneer and non-pioneer Hawaiian Pteridophyta. Bot. Jour. Linn. Soc. In Press.

——— & E. J. KLEKOWSKI, JR. 1970. Spore germination and viability in Pteridophyta: Evolutionary significance of chlorophyllous spores. Biotropica 2: 129–137.

NÄF, U. 1959. Control of antheridium formation in the fern species *Anemia phyllitides*. Nature 184: 798–800.

———. 1960. On the control of antheridium formation in the fern species *Lygodium japonicum*. Proc. Soc. Exp. Biol. 105: 82–86.

———, J. SULLIVAN & M. CUMMINS. 1969. New antheridogen from the fern *Onoclea sensibilis*. Science 163: 1357–1358.

PETERSEN, R. L. & D. E. FAIRBROTHERS. 1973. Allelopathy: gametophytic chemo-antagonism between *Dryopteris* and *Osmunda* or supporting the "weakest link" hypothesis. Amer. Jour. Bot. 60 (4, suppl.): 32.

SAUS, G. L. 1973. Reproductive biology of the fern *Onoclea sensibilis* L. Unpublished master's thesis, Ohio Univ., Athens.

SCHEDLBAUER, M. D. & E. J. KLEKOWSKI, JR. 1972. Antheridogen activity in the fern *Ceratopteris thalictroides* (L.) Brongn. Bot. Jour. Linn. Soc. 65: 399–413.

TATUNO, S. & H. YOSHIDA. 1966. Karyological studies on Osmundaceae. I. Chromosomes of the genus *Osmunda* in Japan. Bot. Mag. (Tokyo) 79: 244–252.

——— & ———. 1967. Karyological studies on Osmundaceae. II. Chromosomes of the genera *Osmundastrum* and *Plenasium* in Japan. Bot. Mag. (Tokyo) 80: 130–138.

VOELLER, B. R. 1964. Antheridogen in ferns. Natl. Rech. Sci. (Paris) 123: 665–684.

WAGNER, W. H., JR. 1970. Evolution of *Dryopteris* in relation to the Appalachians. *In* "The distributional history of the biota of the southern Appalachians, Part. II." Virginia Polytechn. Inst. & St. Univ. Res. Div. Monogr. 2.